

The Impact of Sex Differences on Fear Extinction and Extinction Recall

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A report submitted in partial requirement for the degree of Master of Psychology  
(Clinical) at the University of Tasmania, 2015.

**Statement of Originality**

I declare that this research report is my own work and that, to the best of my knowledge and belief, it does not contain material from published sources without proper acknowledgement, nor does it contain material which has been accepted for the award of any other higher degree or graduate diploma in any university.

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November 20, 2015

## SEX DIFFERENCES IN FEAR EXTINCTION AND RECALL

### **Acknowledgements**

Firstly, I would like to thank Professor Kim Felmingham for your knowledge, support and guidance throughout the past two years. Your passion in the field of psychology has inspired me to continue to learn and grow. I would like to thank Dr Kimberly Norris in helping me recruit participants for my study. Thank you to everyone who willingly participated in my experiment, even though you sat through an ‘uncomfortable, but not painful’ experience.

Thank you to all the friends that I have made in Hobart. I know that I have made lifelong friends in such a short amount of time, and without your friendship and support, it would’ve been a much more challenging move from Sydney. In particular, I would like to thank Tamara, who continues to encourage me and make me laugh. Thank you to my friends in Sydney, who always made time for me despite their busy schedules.

Thank you to my family for being so understanding and supportive of me, despite me leaving home at the ripe old age of 21.

Lastly, I would like to thank Joshua for your amazing support, even though you were a two-hour flight away. You continue to surprise me with your dedication and love.

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Word Count: 8898



### **Abstract**

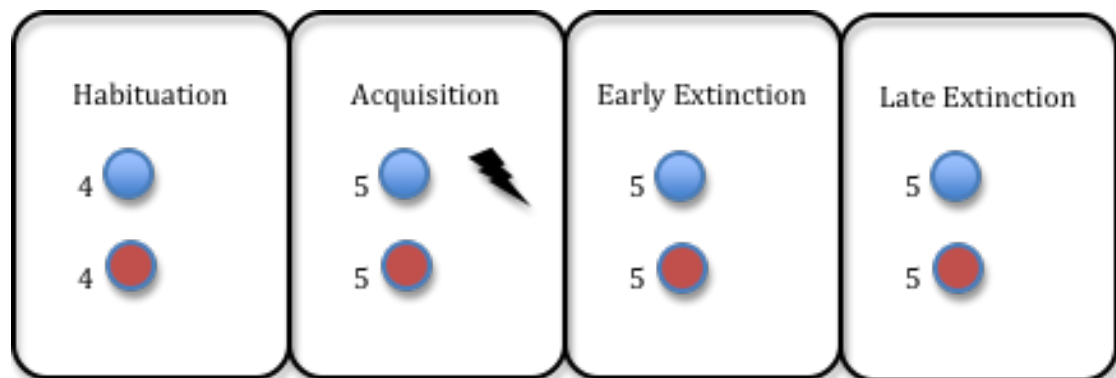
Research indicates that sex differences are observed in the incidence of anxiety disorders, with females more likely to have an anxiety disorder than males. Animal and human studies suggest that gonadal hormones may influence the rate of extinction learning. In particular, studies have indicated that low oestrogen levels in females may impair extinction recall. The aim of the current study was to replicate the findings obtained by previous authors, by investigating sex differences in fear conditioning, fear extinction, and extinction recall, in particular in women with low levels of oestrogen. It was hypothesised that women with low oestrogen would display impaired fear extinction and extinction recall, in comparison to men, which was measured by skin conductance responses (SCR). Twenty-seven participants (fifteen females; twelve males) underwent a differential fear conditioning and fear extinction paradigm. Results revealed no sex differences in fear acquisition or late extinction. However, males displayed greater SCR to the CS- in early extinction, which may reflect greater arousal to uncertainty. Contrary to predictions, no significant sex differences were observed in the early recall phase. However, females displayed a trend for greater SCR responses during late recall, relative to men. These findings provide some support that females with low oestrogen display poorer fear extinction retention, compared to men. In addition, the findings suggest that males display enhanced responses to safety signals during extinction. It is possible that oestrogen may be an important factor in consolidating extinction memories, but further research is required to determine the role of oestrogen in facilitating extinction recall.

An inability to successfully extinguish a conditioned fear may contribute to the occurrence and maintenance of anxiety disorders (Milad et al., 2006).

Epidemiological studies suggest that women have a greater propensity to develop an anxiety disorder than men (Kessler et al., 2005; Lebron-Milad & Milad, 2012; Merz et al., 2012). For example, studies have found that women are more likely to receive a diagnosis of posttraumatic stress disorder (PTSD), have greater severity of PTSD symptoms with a longer duration, and higher ratings of functional impairment (Cover, Maeng, Lebron-Milad, & Milad, 2014). In addition, 60% of individuals who have a diagnosis of generalised anxiety disorders are women, and these women are more likely to have a comorbid psychological disorder (Cover et al., 2014). Clinical research has indicated that sex hormones may play a role in influencing mood in women (Solomon & Herman, 2009). For example, hormonal fluctuations that often occur during premenstrual, postpartum and perimenopausal periods have been linked with higher incidences of depression and anxiety (Douma, Husband, O'Donnell, Barwin, & Woodend, 2005; Solomon & Herman, 2009). Recently there has been convergent animal and human evidence suggesting that low oestrogen levels are associated with impaired fear extinction learning, leading to suggestions that fluctuating levels of oestrogen may be a risk factor in developing anxiety disorders in women (Graham & Milad, 2013; Milad et al., 2006; Milad et al., 2010). However, the link between low levels of oestrogen and impaired fear extinction has originated primarily from the same researchers and this finding requires independent replication from a separate laboratory. Therefore, the aim of this project is to replicate the findings that low oestrogen is associated with impaired fear extinction and extinction recall.

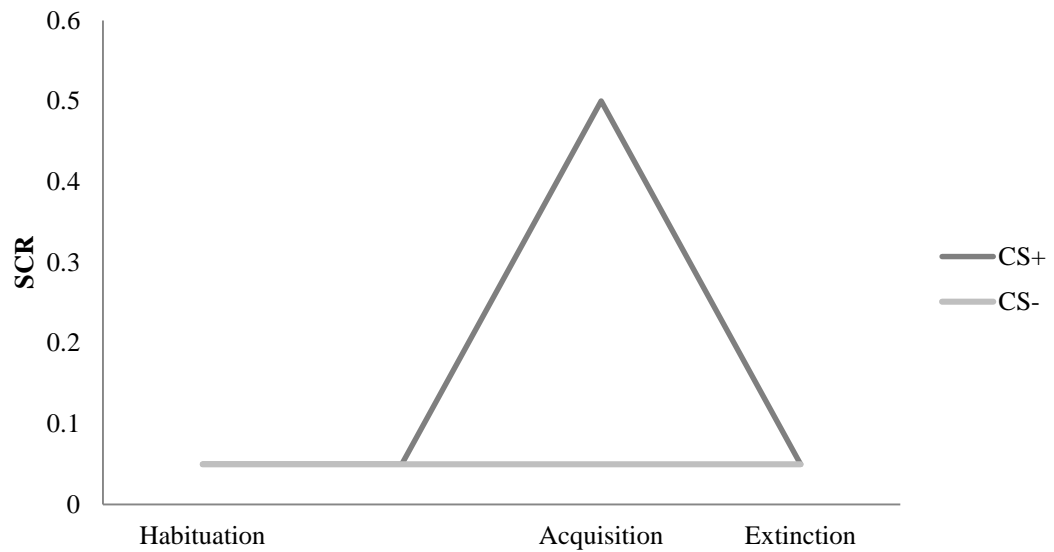
### **Fear Conditioning and Extinction Paradigms**

Past research examining fear extinction has employed a differential fear conditioning and fear extinction paradigm (Chang et al., 2009; Graham & Milad, 2011; Milad et al., 2006; Orr et al., 2000). In Day 1 of this paradigm, in the fear conditioning phase, participants are presented with a neutral conditioned stimulus (CS+), such as a coloured circle, that is paired with an aversive unconditioned stimulus (US), such as a mild shock, which elicits an unconditioned fear response (UR) (see Figure 1 below). A different coloured circle (CS-) is presented randomly interspersed with the CS+, and is never paired with the US and therefore acts as a safety signal.



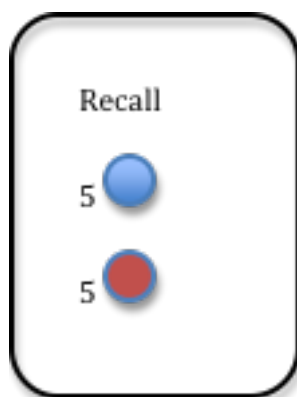
*Figure 1.* Example of a fear conditioning and fear extinction paradigm.

After repeated pairings of the CS+ and US, the participant learns that the CS+ is predictive of the US, and this elicits a conditioned fear response (CR), typically reflected in increased skin conductance responses (SCR). In the fear extinction phase, the CS+ is repeatedly presented without the US, and this reduces and extinguishes the CR (see Figure 2).



*Figure 2.* Example of SCR in the habituation, acquisition, and extinction phase in a fear conditioning and fear extinction paradigm.

In Day 2 of the paradigm (extinction recall), which is identical to the extinction phase, the CS+ and CS- are presented in a random order with no US (see Figure 3 below). In the extinction recall phase, a greater conditioned response to the CS+ reflects poorer extinction retention.



*Figure 3.* Example of the extinction recall phase.

Past research examining fear conditioning and fear extinction has found a consistent pattern in fear responses, such that during the acquisition phase, fear

responses to a CS+ will increase, and during the extinction phase, the conditioned fear responses to a CS+ will decrease (Hermans, Craske, Mineka, & Lovibond, 2006; Orr et al., 2000; Phelps, Delgado, Nearing, & LeDoux, 2004; Vansteenwegen et al., 2005). In humans, one of the most common measures of fear response is skin conductance. Using a differential fear conditioning and fear extinction paradigm, studies have shown increased skin conductance responses (SCR) during the acquisition phase, where a CS+ is paired with a US (Orr et al., 2000; Phelps et al., 2004; Vansteenwegen et al., 2005). This pattern is not observed with the CS-. In the extinction phase, after repeated pairings of the CS+ without a US, SCR diminishes (Orr et al., 2000; Phelps et al., 2004; Vansteenwegen et al., 2005). Similar responses are observed in rats, with increases in the rat's freezing or startle response in the acquisition phase, and reduction in the freezing or startle response in the extinction phase (Milad & Quirk, 2002; Walker, Ressler, Lu, & Davis, 2002).

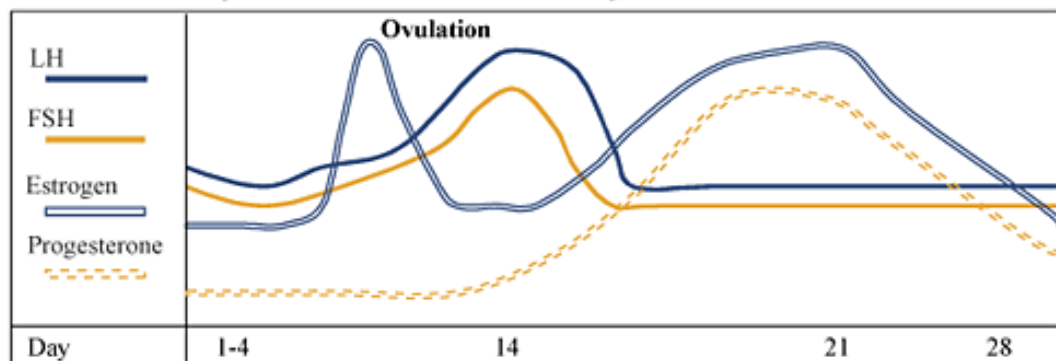
### **Sex Differences in Fear Conditioning and Extinction**

Inconsistent findings have been found in early studies of sex differences in fear conditioning and fear extinction (Andreano & Cahill, 2009; Jackson, Payne, Nadel, & Jacobs, 2006). However, those studies did not control for menstrual phases in female participants. Recent evidence indicates that there are sex differences in fear extinction that may be mediated by hormones, specifically oestrogen levels in women (Graham & Milad, 2013; Lebron-Milad, Graham, & Milad, 2012; Lebron-Milad & Milad, 2012).

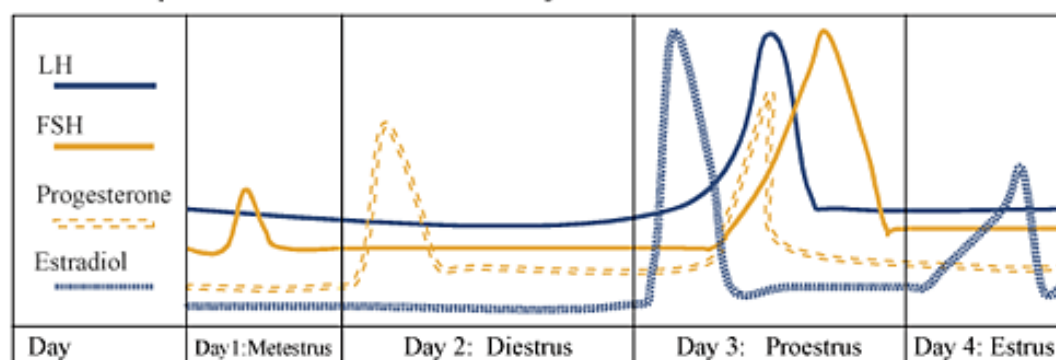
Women in the different phases of the menstrual cycle have varying levels of oestrogen and progesterone (see Figure 4), which may have direct implications on rates of extinction learning (Farage, Osborn, & MacLean, 2008). In the early follicular phase (days 1-7 of the menstrual cycle), women typically have low levels

of oestrogen and progesterone, whereas women in the late follicular phase (days 8-14) have elevated levels of oestrogen and low levels of progesterone (Lebron-Milad, Abbs, et al., 2012; Milad et al., 2006; Poromaa & Gingnell, 2014). In the luteal phase (days 15-28), women display elevated levels of oestrogen and progesterone (Fehring, Schneider, & Raviele, 2006).

### A Human Reproductive Menstrual Cycle



### B Rat Reproductive Menstrual Cycle



*Figure 4.* Oestrogen and progesterone levels in humans and rats across different phases of the menstrual cycle.

### Animal Evidence of Sex Differences in Fear Conditioning and Fear Extinction

In an earlier study conducted by Chang et al. (2009), male rats, normally cycling female rats and ovariectomised female rats underwent a contextual fear conditioning and fear extinction paradigm. Chang et al. (2009) found that male rats

displayed greater levels of freezing following the contextual fear conditioning, relative to normally cycling female rats. Female rats in the proestrus phase, which is characterised by high levels of oestrogen (see Figure 4), and rats in the estrus phase, characterised by mid-levels of oestrogen, demonstrated greater extinction learning relative to male rats (Chang et al., 2009). This suggests that oestrogen may play an important role in mediating differences between male and female extinction learning. In addition, ovariectomised female rats that were injected with oestrogen exhibited greater extinction learning, which provides further evidence to support this idea (Chang et al., 2009).

Using a 3-day fear conditioning and extinction paradigm, male and female rats received fear conditioning on Day 1, fear extinction on Day 2, and were tested their extinction recall on Day 3 (Milad, Iggoe, Lebron-Milad, & Novales, 2009). Rats were tested in two phases of the estrous cycle: the proestrus phase (characterised by high levels of oestrogen and progesterone) and the metestrus phase (characterised by low levels of oestrogen and progesterone). There were differences obtained in the different groups of female rats in the extinction phase in the fear conditioning and fear extinction paradigm (Milad et al., 2009). Rats in the proestrus phase, relative to the metestrus phase displayed lower levels of fear behaviour, such as freezing, in the extinction recall task, which suggests a greater facilitation of extinction memory in the high oestrogen/progesterone phase (Milad et al., 2009). Female rats in the proestrus phase displayed similar levels of extinction recall to male rats (Milad et al., 2009).

In addition, Milad et al. (2009) compared the effect of the administration of oestrogen, progesterone, both oestrogen and progesterone, or vehicle in female rats in the metestrus phase (low levels of oestrogen), on fear conditioning and fear

extinction. The extinction recall task demonstrated that rats that were administered the vehicle exhibited higher amounts of freezing, relative to the other three groups (Milad et al., 2009). This lends further support to the idea that oestrogen has a possible role in the facilitation of extinction recall.

Furthermore, in an experiment conducted by Zeidan et al. (2011), naturally cycling rats in the metestrus phase (characterised by low oestrogen and progesterone) underwent a 3-day conditioning, extinction, and extinction recall paradigm, to examine the effect of having an immediate or a delayed administration of oestrogen after the extinction phase on Day 2 on the extinction recall phase on Day 3. Rats that were administered oestrogen after the extinction phase displayed lower levels of freezing in the recall phase, relative to rats that received vehicle (Zeidan et al., 2011). However, there was no difference between the recall phase in rats that were administered oestrogen or vehicle four hours after the extinction phase (Zeidan et al., 2011).

### **Human Evidence of Sex Differences in Fear Conditioning and Fear Extinction**

In an early study conducted by Milad et al. (2006), a 2 day differential fear conditioning and extinction paradigm was used to examine the effect of sex and menstrual cycle in healthy humans. Specifically, they examined females in the early follicular phase of their menstrual cycle (characterised by low levels of oestrogen), and females in the late follicular phase of their menstrual cycle (characterised by high levels of oestrogen). On the first day, participants were presented with the fear conditioning and fear extinction procedure; on the second day, participants were tested on their extinction recall and fear renewal. A 500ms electrical stimulus was used as the US, which was delivered following the presentation of the CS+ in the fear conditioning procedure (100% reinforcement rate). Milad et al. (2006) found



that men displayed greater fear conditioning responses, exhibited as higher levels of skin conductance responses, relative to both groups of females. Contrary to predictions, women in the early follicular phase demonstrated lower SCR to the CS+ at recall, reflecting greater extinction retention, in comparison to women in the late follicular phase (Milad et al., 2006). There was no significant difference in extinction retention in women in the early follicular phase and men (Milad et al., 2006). However, it should be noted that the level of gonadal hormones in participants were not measured in this study.

In a later study conducted by Milad et al. (2010), the differential fear conditioning and fear extinction paradigm was once again implemented, investigating the effect of oestrogen and sex differences, in healthy humans. However, in this study, women were divided into high and low oestrogen groups using a median split, rather than comparing women on the basis of their phase in their natural menstrual cycle (Milad et al., 2010). Men demonstrated higher levels of skin conductance responses in the acquisition phase, which supports the findings of Milad et al. (2006). In addition, Milad et al. (2010) found that women with low levels of oestrogen displayed a lower level of extinction retention (more fear), relative to men and women with high levels of oestrogen. It is possible that oestrogen may assist in the consolidation of extinction learning, as women with high oestrogen levels exhibit higher levels of extinction memory (Milad et al., 2010). This contradicts the findings of Milad et al. (2006), in which women in the early follicular phase exhibited less SCR to the CS+ at recall. Milad et al. (2010) suggest that the difference in results may be explained by the fact that they had measured the level of gonadal hormones in this study, whereas this was not examined in the study conducted by Milad et al. (2006).

In a study conducted by Zeidan et al. (2011), female participants were separated into high vs low oestrogen groups, and a 2-day fear conditioning and fear extinction paradigm that was identical to previous studies was used to examine the effect of oestrogen on fear extinction. This study showed that female participants with low levels of oestrogen, in comparison to females with high levels of oestrogen, showed higher levels of SCR at extinction recall, reflecting impaired extinction recall (Zeidan et al., 2011). This supports the findings of Milad et al. (2010), and also lends support to the idea that low levels of oestrogen in females may impair the retention of extinction memory.

Additionally, a recent study conducted by Graham and Milad (2013) examined the impact of hormonal contraceptives and oestrogen supplementation on fear extinction recall in women. Research has indicated that combined hormonal contraceptives lead to a reduction in the level of oestrogen in females, leading to a level of oestrogen that is similar to the level of oestrogen exhibited in females in the early follicular phase (Pluchino et al., 2009). Graham and Milad (2013) found that women with low levels of oestrogen and women on hormonal contraceptives displayed greater SCR at extinction recall, relative to women with high levels of oestrogen. Additionally, Graham and Milad (2013) administered oestradiol to women in the low follicular phase 30 minutes prior to extinction training and found that they displayed lower levels of SCR (less fear) at extinction recall, relative to women in the early follicular phase who received placebo. This supports the findings obtained in rat studies, whereby rats that received an administration of oestrogen did not display impairments in extinction recall, compared to control (Graham & Milad, 2013). This provides further evidence for the idea that low oestrogen may impair the consolidation of fear extinction at extinction recall.

## **The Current Study**

Research has demonstrated that sex differences are observed in the incidence of anxiety disorders, with females being more likely than males to have an anxiety disorder (Kessler et al., 2005; Lebron-Milad, Abbs, et al., 2012). Despite these differences, limited studies have specifically examined the sex differences in fear conditioning and fear extinction, which is the process believed to underlie the development and treatment of anxiety disorders. The limited research suggests that oestrogen may play an important role in influencing the degree of fear extinction and retention of extinction memory (Graham & Milad, 2013; Milad et al., 2010; Zeidan et al., 2011). In particular, studies suggest that low levels of oestrogen may impair the retention of extinction memory, as female rats and female humans with low levels of oestrogen exhibit greater fear responses at extinction recall, relative to female rats and females humans with high levels of oestrogen, and male rats and male humans (Graham & Milad, 2013; Milad et al., 2009; Zeidan et al., 2011). Furthermore, when rats or humans with low levels of oestrogen are administered oestrogen prior to extinction training, they are able to increase their ability to retain their extinction memories (Graham & Milad, 2013; Milad et al., 2009; Zeidan et al., 2011). Thus, oestrogen may be an important mediator of extinction memories. However, it should be noted that there have only been a limited number of studies examining these sex differences in fear extinction, which are also conducted by the same authors. Therefore independent replication of these findings are important as these findings may have potentially important clinical implications.

Therefore, the primary aim is to examine differences in the rate of fear extinction learning between males and females in the early follicular phase by employing a fear conditioning and fear extinction paradigm. A secondary aim of the

current study is to examine sex differences in delayed extinction recall, as preliminary studies have found the effects of oestrogen predominantly in extinction recall rather than extinction learning.

It is hypothesised that women in the early follicular phase (associated with low levels of oestrogen and progesterone) of their menstrual cycle will display impaired fear extinction, in comparison to men. This will be demonstrated in higher levels of SCR amplitude during the extinction phase to the CS+. It is also hypothesised that in the recall phase, women in the early follicular phase will display impaired fear extinction retention, relative to men. This will be indicated by a greater recovery of fear in females (indexed by greater SCR to CS+ at recall), relative to men.

## **Method**

### **Participants**

Twenty-seven participants were recruited for the study from Psychology 1 and students received course credit for their participation. Fifteen participants were women who were in the early follicular phase of their menstrual cycle (determined as being between days 2-7 of their menstrual cycle ( $n = 8$ ), or in the sugar pill week of their contraceptive pill ( $n = 7$ ), as research has shown similar levels of oestrogen as normally cycling women in the early follicular phase (Pluchino et al., 2009))<sup>1</sup>, and twelve participants were male. Salivary measures were used to measure oestrogen and progesterone levels and confirm menstrual phase position. All participants ranged from 18 to 45 years of age to control for menopause. Participants were excluded if they were taking hormonal contraceptives other than the contraceptive

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<sup>1</sup> Although recruitment focused on women not on contraceptives, due to considerable difficulty recruiting such participants, we needed to include women on contraceptives, but only while they were on the sugar pill phase.

pill, other medications, were pregnant, or if they reported hypertension or any psychological, neurological, or cardiovascular disorders. Participants refrained from alcohol consumption, illicit drug use and excessive exercise 24 hours prior to participation in the experiment. In addition, participants refrained from drinking caffeine or smoking three hours prior to the experiment, and refrained from eating one hour prior to the experiment.

### **Design**

For the fear acquisition and extinction study, a 2 (Group: male, early follicular women) x 2 (CS type: CS+, CS-) x 4 (Trial: 1, 2, 3, 4) mixed factorial design was employed for the Habituation phase. A 2 (Group: male, early follicular women) x 2 (CS type: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial design was employed for the Acquisition, Early-Extinction, and Late-Extinction phase in Session 1. The dependent variable in Session 1 comprised SCR amplitude. In accordance with previous literature, a percent recovery of fear value was calculated to index extinction recall in Session 2 (Graham & Milad, 2013).

### **Materials**

**Fear conditioning and extinction paradigm.** The current study used a differential fear conditioning and extinction paradigm adapted from previous studies (Inslicht et al., 2013; Milad et al., 2006; Zeidan et al., 2011). The CS+ and CS- comprised a blue or red circle, with colours counterbalanced between participants. The US was a 500-ms mild electric shock produced by the Powerlab 16/35 Stimulus Isolator, and was delivered to the dominant hand by the Powerlab 16/35 Recording Bare Electrode. Skin conductance response amplitude was measured through the use of the PowerLab 16/35 GSR Amp and the GSR Finger Electrodes, which was attached to the non-dominant hand on the first and third fingers. The differential fear

conditioning extinction paradigm was presented using Inquisit 3.0.6.0 and Labchart 7.3.7 was used to obtain skin conductance response amplitude.

**Saliva samples.** Salivary samples were obtained using Salimetrics assay kits and analysed for oestrogen and progesterone level via commercially available standardised techniques in the pathology laboratory at Macquarie University, and to confirm the early follicular phase in women<sup>2</sup>.

**Depression, Anxiety and Stress.** The Depression Anxiety Stress Scales (DASS-21; (Lovibond & Lovibond, 1995)) is a 21-item scale (see Appendix A1) that was administered to rate mood over the past week. The DASS-21 uses a 4-point Likert scale in response to statements such as “I found it hard to wind down”, ranging from 0 (*did not apply to me at all*) to 3 (*applied to me very much, or most of the time*). The DASS-21 displayed high internal consistency in the depression, anxiety and stress subscales, with Cronbach’s alphas of .94, .87 and .91 in each scale respectively (Antony, Bieling, Cox, Enns, & Swinson, 1998).

**Emotion Dysregulation.** The Difficulties in Emotion Regulation Scale (DERS; (Gratz & Roemer, 2004)) is a 36-item questionnaire (see Appendix A2) was used to assess emotion dysregulation, using a 5-point scale with 1 being ‘*almost never*’ and 5 being ‘*almost always*’. An example included “When I’m upset, I feel guilty for feeling that way”. The DERS demonstrated high internal consistency with a Cronbach’s alpha of .93 (Gratz & Roemer, 2004).

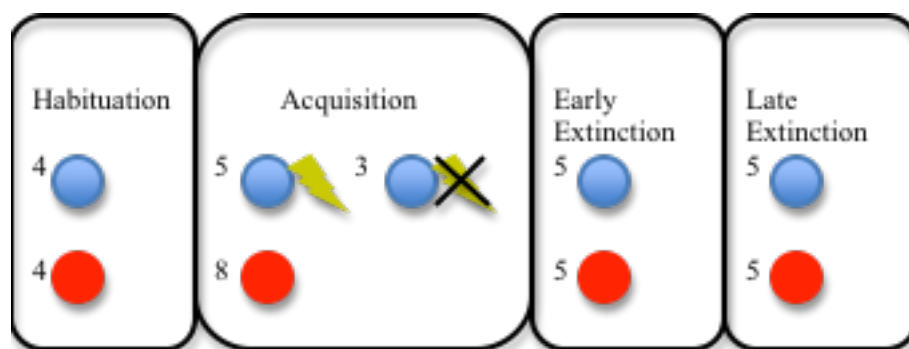
**Health Questionnaire.** A health questionnaire was used to assess medication, alcohol and drug use (see Appendix A3). In addition, it examined exercise, smoking, and the presence of neurological or psychological disorders.

## Procedure

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<sup>2</sup> Although salivary samples were obtained, they are yet to be analysed as it is not within the scope of the study.

Ethics approval was obtained from the Tasmanian Social Sciences Human Research Ethics Committee (see Appendix B1). On arrival, participants were given a participant information sheet (see Appendix B2) and informed consent was obtained (see Appendix B3). Baseline saliva samples were taken using Salimetrics saliva collection tubes using a standardized passive drool method. Participants were then given the DASS-21, DERS and CCQ-M questionnaires to complete (see Appendix A). The US electrode was attached to the participant, and the participant determined the intensity of the shock, by administering the lowest level (2mA) and increasing the intensity until the participant reported the shock to be uncomfortable but not painful. The skin conductance response recording disks were attached to the participant's fingers. Participants were told to remain still throughout the experiment in order to minimise movement artefact responses in the recording of skin conductance responses. Following this, participants completed the fear conditioning and extinction task. There were three phases in this task: habituation, acquisition, and extinction. In the habituation phase, eight coloured circles (four blue, four red) were presented in a randomised order with no shock administered (as shown in Figure 5 below). Before commencing the habituation phase, participants were informed that they were not to receive an electric shock.

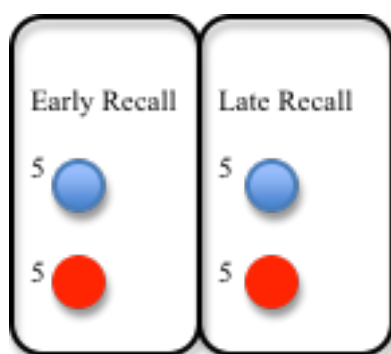


*Figure 5.* Session 1 of the differential conditioning and extinction paradigm.

In the acquisition phase, sixteen coloured circles (8 blue, 8 red) were presented in a randomised order. The US was administered immediately after the CS+ was presented with a 62.5% partial reinforcement schedule (5 out of 8 of the CS+ was paired with the US), in order to prevent rapid habituation to the CS+ during the acquisition phase. The extinction phase, which was divided into an early and a late sub-phase followed the acquisition phase. In each extinction sub-phase, ten coloured circles (5 blue, 5 red) were presented in a randomised order with no shock administered. Before commencing the acquisition and extinction phase, participants were informed that they ‘may or may not be administered with an electric shock’.

Following the completion of the experiment, participants completed a questionnaire that examined whether they had understood CS+/US contingency.

For the delayed recall measure, a second testing session was conducted 24 hours following the initial session. In this session, SCR recording disks and the US electrode were attached to the participant’s hand and they completed the extinction phase of the task a second time (see Figure 6 below). SCR was recorded as per session 1. Participants were debriefed after the completion of the experiment.



*Figure 6.* Session 2 of the differential conditioning and extinction paradigm.

### **Analysis**

The SCR amplitude for each CS trial was indicative of the change from skin conductance level (SCL) baseline due to the presentation of the CS. Each CS was



displayed for 12 seconds, with an inter-trial interval ranging from 12 seconds to 21 seconds (mean of 16 seconds). Skin conductance levels were indexed 2 seconds prior to the display of the CS, 12 seconds while the CS was displayed and also 6 seconds after the US was administered. Baseline SCL for each participant was calculated by averaging the SCL amplitude 2 seconds prior to the CS presentation in the Habituation phase. SCR amplitude was calculated by obtaining the highest skin conductance level (SCL) during the 12 second CS presentation and subtracting the mean SCL of the 2 seconds prior to the presentation of the CS. The SCR amplitude was indicative of the change from the baseline SCL following the CS presentation. In order to reduce reduce heteroscedasticity, the SCR data was square-root transformed (Milad et al., 2010).

Data analysis for the fear acquisition and extinction study was conducted using 2 (Group: male, early follicular women) x 2 (CS: CS+, CS-) x (Trial: 1, 2, 3, 4) mixed factorial ANOVA in the Habituation phase, 2 (Group: male, early follicular women) x 2 (CS: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial ANOVA in the Acquisition phase, 2 (Group: male, early follicular women) x 2 (CS: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial ANOVA in the Early Extinction and Late Extinction phase. For the Early and Late Recall phase, a percent recovery of fear value was calculated in order to control for the effect of conditioning (Graham & Milad, 2013). In this measure, the average SCR for each participant in the early and late recall phase were divided by their largest SCR to the CS+ in the acquisition phase. This was then multiplied by 100 to obtain the percent recovery of fear. A one-way ANOVA was used to compare percent recovery of fear in the Early and Late Extinction Recall phase.

## Results

### Demographic and Clinical data

A summary of demographic and clinical data is presented in Table 1. As shown in Table 1, males and females did not differ significantly with respect to age, body mass index (BMI), hours since [awakening](#), quality of sleep, or time of day of the experiment. There were no significant differences between males and females on their subjective ratings of depression, anxiety and stress, as measured by the DASS-21. Additionally, there were no significant differences between males and females in their ability to regulate their emotions, as measured by the DERS. There was no significant difference between males and females on the level of shock intensity selected.

Table 1

*Means, Standard Deviations and F-tests for males and females for demographics*

	Males <i>M</i> ( <i>SD</i> )	Females <i>M</i> ( <i>SD</i> )	<i>F</i>	<i>p</i>	$\eta p^2$
Age	24.92 years (6.10)	25.53 years (6.89)	0.06	.810	.002
BMI	21.67 (2.42)	20.80 (2.98)	0.66	.423	.026
Hours since wakening	6.25 (3.14)	7.07 (2.63)	0.54	.469	.021
Quality of sleep	6.83 (2.12)	6.60 (2.44)	0.07	.796	.003
Time of experiment	14:30 (2:35)	14:31 (2:07)	0.00	.998	<.001
DASS-21 Depression	3.75 (4.88)	3.13 (3.02)	0.16	.690	.006
DASS-21 Anxiety	3.33 (5.42)	3.87 (3.54)	0.10	.760	.004

DASS-21 Stress	4.83 (3.97)	5.87 (3.20)	0.56	.461	.022
DERS	139.63 (177.44)	90.00 (20.24)	1.17	.291	.045
Shock intensity	2.06 (0.22)	1.80 (0.53)	2.50	.126	.091
Baseline SCL	8.21 (4.70)	7.05 (4.44)	0.43	.571	.017

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Note: M = Mean, SD = Standard Deviation

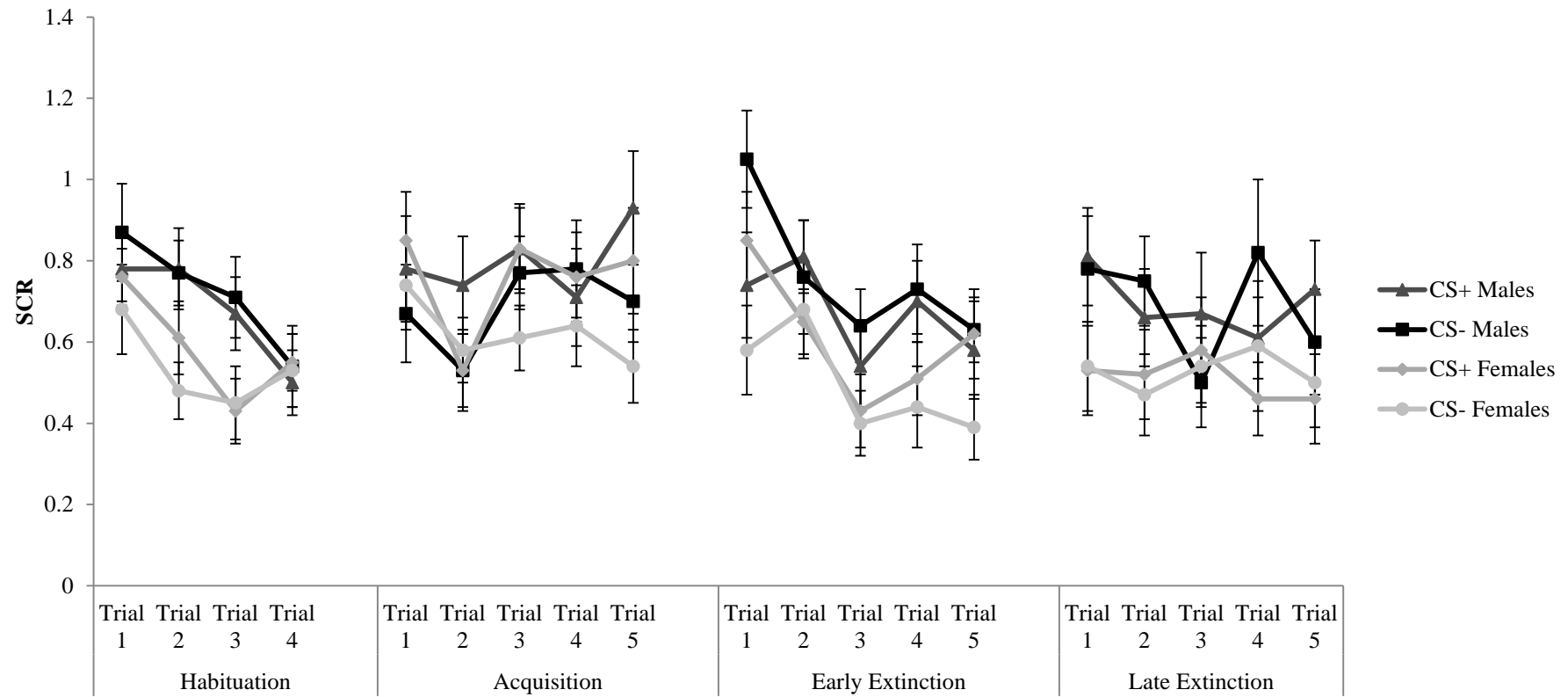
### Skin Conductance

**Baseline Skin Conductance.** As shown in Table 1, there was no significant difference between males' and females' baseline SCL.

**Habituation.** A 2 (Sex: Male, Female) x 2 (Condition: CS+, CS-) x 4 (Trial: 1, 2, 3, 4) mixed factorial ANOVA found that the main effects of Condition ( $F(1, 25) = 0.09, p = .772, \eta^2 = .003$ ) and Sex ( $F(1, 25) = 2.63, p = .118, \eta^2 = .095$ ) were not significant. The main effect of Trial was significant,  $F(3, 75) = 10.06, p < .001, \eta^2 = .287$ . Sidak-adjusted pairwise comparisons revealed that there was a significant reduction in SCR from Trial 1 ( $M = .76$ ) to Trial 4 ( $M = .53, p < .001, 95\% \text{ CI } [0.10, 0.37]$ ). The main effect of Trial was moderated by a significant Sex and Trial interaction,  $F(3, 75) = 3.56, p = .018, \eta^2 = .125$  (see Figure 7). Sidak-adjusted pairwise comparisons broken down by Sex found that males' mean SCR was significantly higher than females' at Trial 2 ( $p = .043, 95\% \text{ CI } [0.01, 0.45]$ ) and at Trial 3 ( $p = .042, 95\% \text{ CI } [0.01, 0.49]$ ), but not at Trial 1 ( $p = .239, 95\% \text{ CI } [-0.09, 0.33]$ ) and Trial 4 ( $p = .805, 95\% \text{ CI } [-0.23, 0.18]$ ). Sidak-adjusted pairwise comparisons broken down by Trial revealed that males' SCR was not significantly higher from Trial 1 ( $M = .82$ ) to Trial 2 ( $M = .77, p = .972, 95\% \text{ CI } [-0.13, 0.23]$ ), Trial 2 to Trial 3 ( $M = .69, p = .663, 95\% \text{ CI } [-0.09, 0.26]$ ) and Trial 3 to Trial 4 ( $M = .52, p = .268, 95\% \text{ CI } [-0.07, -0.41]$ ). In contrast, females' SCR was trending higher from Trial 1 ( $M = .70$ ) to Trial 2 ( $M = .55, p = .066, 95\% \text{ CI } [-0.01, 0.32]$ ),

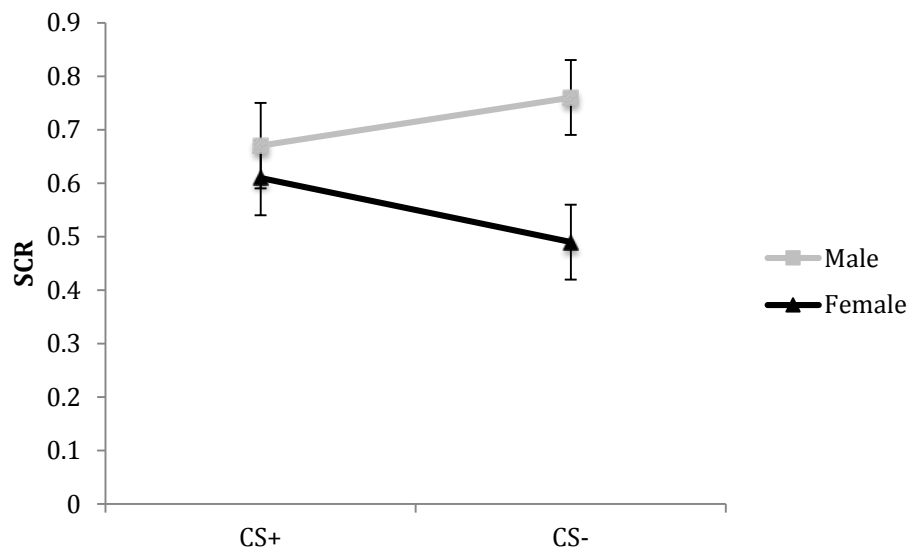
but not significantly different at Trial 2 to Trial 3 ( $M = .44, p = .312, 95\% \text{ CI } [-0.05, 0.26]$ ), and Trial 3 to Trial 4 ( $M = .54, p = .707, 95\% \text{ CI } [-0.11, 0.31]$ ). The three-way interaction between Sex, Trial, and Condition was non-significant,  $F(3,75) = 0.36, p = .785, \eta^2 = .019$ . Additionally, the interactions between Sex and Condition, and Trial and Condition were not significant ( $p > .05$ , see Table C1 in Appendix C for details of non-significant interactions).

**Acquisition.** A 2 (Sex: Male, Female) x 2 (Condition: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial ANOVA found a significant main effect of Condition,  $F(1, 25) = 4.89, p = .036, \eta^2 = .164$ , indicating that overall, participants' SCR to the CS+ ( $M = .78$ ) was significantly higher than the participants' SCR to the CS- ( $M = .66$ ). There was a main effect at trend level for Trial,  $F(4, 100) = 2.42, p = .054, \eta^2 = .088$  (see Figure 7). Sidak-adjusted pairwise comparisons demonstrated that Trial 1 ( $M = .76$ ) was trending higher than Trial 2 ( $M = .59, p = .060, 95\% \text{ CI } [0.00, 0.34]$ ). Trial 2 was significantly lower than Trial 3 ( $M = .76, p = .015, 95\% \text{ CI } [-0.31, -0.02]$ ). Trial 3 was not significantly different to Trial 4 ( $M = .72, p = 1.000, 95\% \text{ CI } [-0.16, 0.23]$ ), and Trial 4 was not significantly different to Trial 5 ( $M = .75, p = 1.000, 95\% \text{ CI } [-0.21, 0.15]$ ). The main effect of Sex was non-significant,  $F(1, 25) = 0.41, p = .530, \eta^2 = .016$ . The three-way interaction between Sex, Trial, and Condition was non-significant,  $F(4,100) = 1.30, p = .274, \eta^2 = .050$ . Additionally, the interactions between Sex and Condition, Sex and Trial, and Trial and Condition were not significant and therefore were not explored further (see Table C2 in Appendix C for details of non-significant interactions).



*Figure 7.* SCR for males and females for each CS in the habituation, acquisition, early extinction, and late extinction phase. Error bars indicate standard errors.

**Early Extinction.** A 2 (Sex: Male, female) x 2 (Condition: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial ANOVA found a significant main effect of Trial,  $F(4,100) = 7.75, p < .001, \eta p^2 = .237$  (see Figure 7). Sidak-adjusted pairwise comparisons demonstrated that participants' SCR was significantly higher from Trial 2 ( $M = .72$ ) to Trial 3 ( $M = .50, p = .005, 95\% \text{ CI } [.05, .39]$ ), but was not significantly different from Trial 1 ( $M = .80$ ) to Trial 2 ( $p = .963, 95\% \text{ CI } [-.13, .27]$ ), Trial 3 to Trial 4 ( $M = .60, p = .809, 95\% \text{ CI } [-.30, .11]$ ), or Trial 4 to Trial 5 ( $M = .56, p = .998, 95\% \text{ CI } [-.14, .22]$ ). There was no significant main effect of Sex ( $F(1, 25) = 0.13, p = .772, \eta p^2 = .005$ ) or Condition ( $F(1, 25) = 3.14, p = .089, \eta p^2 = .112$ ).



Note: Error bars are 95% confidence intervals.

*Figure 8.* SCR for CS+ and CS- condition for males and females in the early extinction phase.

The interaction between Sex and Condition was significant,  $F(1,25) = 4.83, p = .037, \eta p^2 = .162$ . As seen in Figure 8 above, Sidak-adjusted pairwise comparisons broken down by Sex found that males' mean SCR ( $M = .76$ ) was significantly higher

than females' mean SCR in the CS- condition ( $M = .49, p = .011, 95\% \text{ CI } [.07, .47]$ ) but was not significantly higher in the CS+ condition ( $p = .593, 95\% \text{ CI } [-.17, .29]$ ). Sidak-adjusted pairwise comparisons broken down by Condition found that males' mean SCR was not significantly different between conditions ( $p = .227, 95\% \text{ CI } [-.24, .06]$ ). Female's mean SCR was significantly higher at trend level in the CS+ condition than the CS- condition ( $p = .067, 95\% \text{ CI } [-.01, .25]$ ). The interaction between Sex and Trial ( $F(4, 100) = .44, p = .780, \eta p^2 = .017$ ) and Trial and Condition ( $F(4, 100) = 0.37, p = .833, \eta p^2 = .014$ ) were not significant and therefore not explored further.

The three-way interaction between Sex, Trial, and Condition was significant,  $F(4,100) = 2.99, p = .022, \eta p^2 = .107$ . Break-down two-way ANOVAs for the Sex by Condition interaction for Trial 1 did not find a significant Condition effect or Sex effect (see Table C3 in Appendix C) but did find a significant Sex by Condition interaction ( $F(1,25) = 9.47, p = .005, \eta p^2 = .275$ ). As can be seen in the early extinction phase panel of Figure 7, post-hoc pairwise comparisons found no significant sex differences to CS+ at Trial 1 ( $p = .520, 95\% \text{ CI } [-.49, .25]$ ) but found that males' SCR was greater than females to CS- on Trial 1 ( $p = .005, 95\% \text{ CI } [.17, .85]$ ). Break-down two-way ANOVAs for Trial 2, Trial 3, Trial 4 and Trial 5 found no significant Condition effect, Sex effect, or Condition by Sex effects (see Table C3 in Appendix C).

Break-down two-way ANOVAs for the Sex by Trial interaction in the CS+ condition revealed no significant Sex effect ( $F(1, 25) = 0.29, p = .593, \eta p^2 = .012$ ), and no significant Sex by Trial effect ( $F(4, 100) = 1.46, p = .221, \eta p^2 = .055$ ). There was a significant Trial effect ( $F(4,100) = 4.94, p = .001, \eta p^2 = .164$ ) in the CS+ condition, with Sidak-adjusted pairwise comparisons indicating that

participants' SCR significant dropped from Trial 2 ( $M = .73$ ) to Trial 3 ( $M = .48$ ,  $p < .001$ , 95% CI [.11, .39]), but was not significantly different from Trial 1 ( $M = .80$ ) to Trial 2 ( $p = .996$ , 95% CI [-.17, .30]), Trial 3 to Trial 4 ( $M = .61$ ,  $p = .716$ , 95% CI [-.36, .11]), or Trial 4 to Trial 5 ( $M = .60$ ,  $p > .999$ , 95% CI [-.25, .26]). Break-down two-way ANOVAs for the Sex by Trial interaction in the CS- condition found a significant Sex effect, with males' SCR being higher than females ( $F(1,25) = 7.53$ ,  $p = .011$ ,  $\eta p^2 = .231$ ). There was no significant Sex by Trial effect ( $F(1, 5) = .92$ ,  $p = .348$ ,  $\eta p^2 = .035$ ). A significant Trial effect was also observed in the CS- condition ( $F(4,100) = 4.39$ ,  $p = .003$ ,  $\eta p^2 = .146$ ), with Sidak-adjusted pairwise comparisons demonstrating that Trial 1 ( $M = .80$ ) was significantly higher than Trial 3 ( $M = .52$ ,  $p = .018$ , 95% CI [.03, .52]), and Trial 1 was significantly higher than Trial 5 ( $M = .51$ ,  $p = .001$ , 95% CI [.09, .48]). There were no significant differences between Trial 1 and Trial 2 ( $M = .72$ ,  $p = .995$ , 95% CI [-.21, .37]), Trial 2 to Trial 3 ( $p = .316$ , 95% CI [-.08, .47]), Trial 3 to Trial 4 ( $M = .39$ ,  $p = .996$ , 95% CI [-.33, .19]), or Trial 4 to Trial 5 ( $p = .990$ , 95% CI [-.19, .34]).

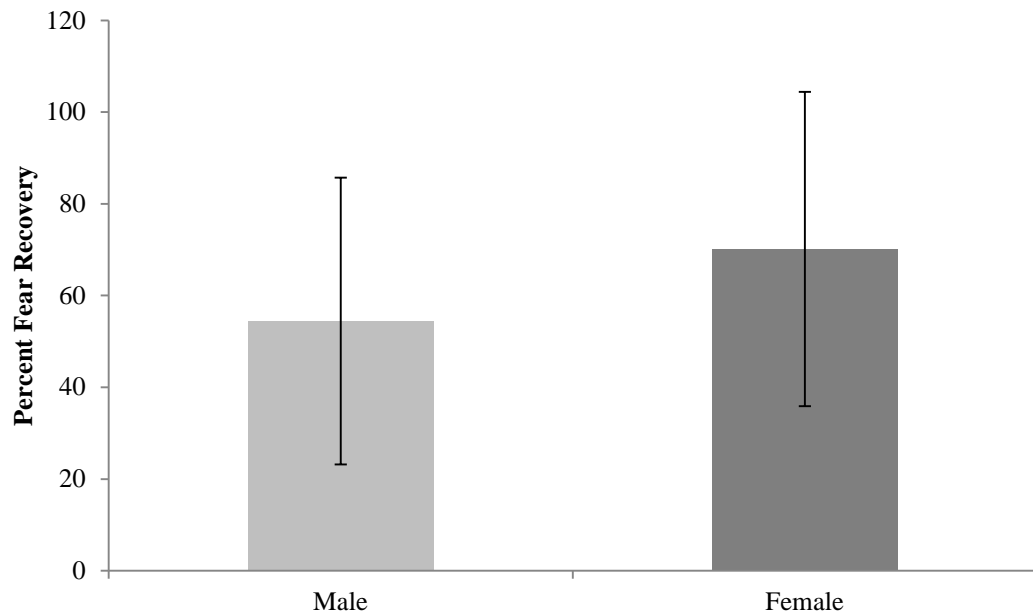
Break-down two-way ANOVAs for the Condition by Trial interaction for females did not find a significant effect of condition, but found a significant Trial effect ( $F(4,56) = 5.60$ ,  $p = .001$ ,  $\eta p^2 = .286$ ). The significant Trial effect was subsumed by a trend in the Trial by Condition effect ( $F(4,56) = .069$ ,  $\eta p^2 = .141$ ). Post-hoc pairwise comparisons found that in Trial 1, females' SCR was trending higher for the CS+ condition than CS- condition ( $p = .057$ , 95% CI [-.01, .64]). Females' SCR was also significantly higher in the CS+ condition at Trial 5 ( $p = .028$ , 95% CI [.03, .43]). There were no significant differences between conditions in Trial 2, Trial 3, or Trial 4 (see Table C4 in Appendix C).



Break-down two-way ANOVAs for the Condition by Trial interaction for males did not find a significant Condition effect ( $F(1, 11) = 1.98, p = .187, \eta p^2 = .153$ ) or Condition by Trial effect ( $F(4, 44) = 1.22, p = .317, \eta p^2 = .100$ ). There was a significant Trial effect,  $F(4, 44) = 3.02, p = .028, \eta p^2 = .215$ . Post-hoc pairwise comparisons indicated that SCR trended higher at Trial 1 ( $M = .89$ ) compared to Trial 5 ( $M = .60, p = .085, 95\% \text{ CI } [-.03, .61]$ ). There were no significant differences observed between Trial 1 and Trial 2 ( $M = .78, p = .952, 95\% \text{ CI } [-.22, .44]$ ), Trial 2 and Trial 3 ( $M = .59, p = .294, 95\% \text{ CI } [-.09, .47]$ ), Trial 3 and Trial 4 ( $M = .72, p = .977, 95\% \text{ CI } [-.55, .30]$ ) or Trial 4 to Trial 5 ( $p = .967, 95\% \text{ CI } [-.24, .47]$ ).

**Late Extinction.** A 2 (Sex: Male, Female) x 2 (Condition: CS+, CS-) x 5 (Trial: 1, 2, 3, 4, 5) mixed factorial ANOVA found no significant main effect of Sex ( $F(1, 25) = 1.92, p = .178, \eta p^2 = .071$ ), Trial ( $F(2.29, 57.36) = 0.65, p = .544, \eta p^2 = .025$ ) following a greenhouse geisser correction, or Condition ( $F(1, 25) = 0.02, p = .882, \eta p^2 = .001$ ) (see Figure 7). All other interaction effects were non-significant ( $p > .05$ , see Table C5 in Appendix C for details of non-significant interactions).

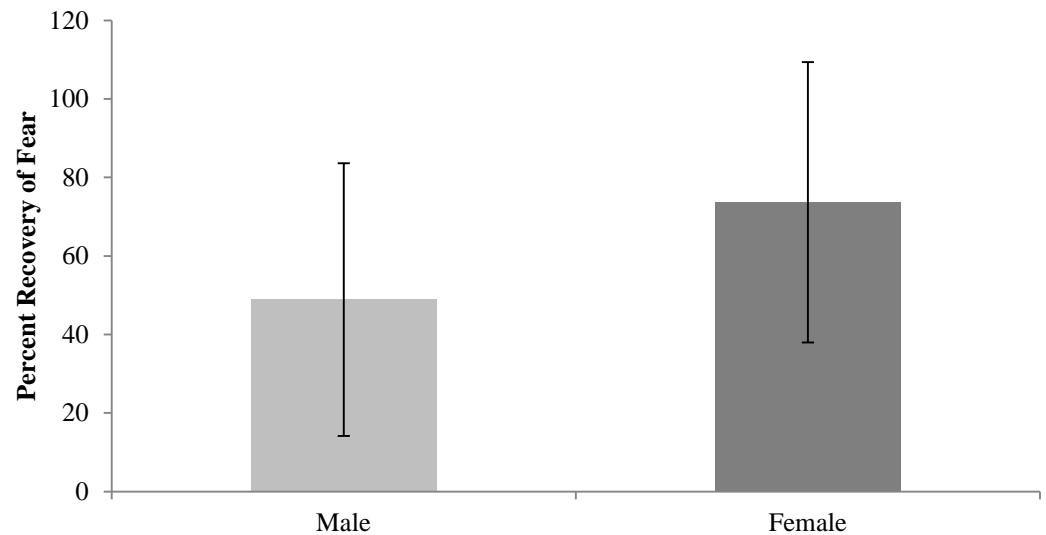
**Extinction Recall Early.** Extinction recall data for the early extinction recall phase was calculated by obtaining a percent recovery of fear, which controls for the conditioning effect. This was calculated by obtaining an average SCR to the CS+ for each participant in the early recall phase which was divided by each participant's largest SCR to the CS+ in the acquisition phase, as according to Graham and Milad (2013). This value was then multiplied by 100 to obtain the percent recovery of fear. A one-way ANOVA found no significant differences in the percent recovery of fear in males ( $M = 54.43$ ) and females ( $M = 70.14$ ) in the early recall phase for CS+,  $F(1, 25) = 1.51, p = .230, \eta p^2 = .057$  (see Figure 9).



Note: Error bars are 95% confidence intervals.

*Figure 9.* Percent fear recovery in the early recall phase for CS+ in males and females.

**Extinction Recall Late.** A percent recovery of fear value was also obtained for the late extinction recall phase, whereby an average SCR to the CS+ for each participant in the late recall phase was divided by each participant's largest SCR to the CS+ in the acquisition phase. This value was then multiplied by 100. A one-way ANOVA found a trend towards significance with females demonstrating a higher percent recovery of fear ( $M = 73.66$ ) than males ( $M = 48.88$ ,  $F(1,25) = 3.29$ ,  $p = .082$ ,  $\eta p^2 = .116$ ) (see Figure 10).



Note: Error bars are 95% confidence intervals.

*Figure 10.* Percent fear recovery in the late phase for CS+ in males and females.

## Discussion

The aim of the current study was to replicate the findings of low oestrogen levels being associated with impaired fear extinction (Graham & Milad, 2013) by comparing SCR during fear conditioning, fear extinction, and extinction recall in women in the low oestrogen, early follicular phase of the menstrual cycle compared to men. Results revealed no significant sex differences across trial or condition in fear acquisition. In the early extinction phase, males displayed greater SCR in initial trials to the CS- than females. There were no significant sex differences obtained in the percent recovery of fear to the CS+ in the early recall phase as predicted, but a trend was found in the late recall phase that females displayed a greater recovery of fear relative to men. These findings provide some support that females with low oestrogen display reduced fear extinction retention compared to males, but also suggest that males display enhanced reactivity to safety signals during extinction, which may reflect a generalized arousal or response to uncertainty.

## Sex Differences in Fear Conditioning and Fear Extinction

**Habituation.** There were no differences in SCR to the CS+ and CS- in the habituation phase. This was expected, as the participants had not yet learnt the association between specific stimuli and the shock. There was main effect of Trial, suggesting that participants had habituated to the stimuli. This main effect was moderated by a Sex by Trial interaction, which demonstrated that male's SCR were higher than females at only Trial 2 and Trial 3, and not Trial 1 or Trial 4. In addition, female's SCR trended higher only from Trial 1 to Trial 2. However, this interaction does not appear to be meaningful, and it is possible that these observations are best explained by the participant's uncertainty about receiving or not receiving a shock.

**Acquisition.** As expected, there was a significant Condition effect during the acquisition phase with participants exhibiting a greater SCR to the CS+ relative to the CS-. This suggests that fear conditioning successfully occurred, as differential responses to the stimuli were observed in the acquisition phase. There was a trend effect of Trial, with Trial 1 trending higher than Trial 2, Trial 2 being significantly lower than Trial 3, but no differences between Trial 3 to Trial 4, or Trial 4 or Trial 5. In addition, there was no significant interaction between Condition and Trial, which suggests that differential responses to the stimuli did not significantly increase across trials. The non-significant interaction may be attributed to a 62.5% reinforcement rate, as participants were shocked five out of eight times in the acquisition phase, in a random order. As such, participants may have heightened uncertainty about whether they would be shocked with the next stimuli, and as such, display high levels of SCR with each presentation of each stimuli.

Due to inconsistent evidence in sex differences in the fear acquisition phase, a directional hypothesis was not predicted. There was no main effect of Sex, or any

interaction effects observed in the fear acquisition phase, which indicates that there were no differences between males and females in their SCR. Previous research in fear conditioning in humans have found that males are more reactive than women during the acquisition phase, i.e., males display greater SCR than females during acquisition (Milad et al., 2006; Milad et al., 2010). However, a study conducted by Zeidan et al. (2011) found no sex differences in the acquisition phase, which is consistent to the findings of our study. A possible explanation for the differences between the SCR in males and females could be the reinforcement rate, as Milad et al. (2006) and Milad et al. (2010) utilised a 100% reinforcement rate, whereas Zeidan et al. (2011) used a 62.5% reinforcement rate, which was also used in the current study.

**Extinction.** Any Sex or Condition or Trial effects or interactions were observed in the early rather than the late extinction phase. In the early extinction phase, there was a significant Trial effect, indicating that participant's SCR reduced across trials. Further, there was no significant condition main effect, suggesting there was no differential SCR to the CS+ and CS- stimuli during early extinction. Taken together, these effects suggest that fear extinction learning has taken place, as there is no longer a discrimination between CS+ and CS-. A Condition by Trial interaction would be the strongest evidence of extinction learning, but this interaction was not observed in the study. However, there was a significant Sex by Condition by Trial interaction effect observed. It was hypothesised that women in the early follicular phase of their menstrual cycle would display impaired fear extinction in comparison to men, which is reflected in higher levels of SCR to the CS+.

The significant three way interaction (Sex by Condition by Trial interaction) found in the early extinction phase revealed an interesting pattern of sex differences.

In line with hypotheses, there was a trend for a Condition by Trial effect in women, but not in men. Pairwise analyses revealed that in women, there was a trend for greater SCR to CS+ compared to CS- on the initial trial of extinction, and this difference became significant at Trial 5 of extinction. In contrast, there was no significant Condition by Trial interaction effect in men. Whilst this must be interpreted cautiously as it is only at trend level and a small effect size, this finding is in line with predictions as it suggests that women continue to react more to the CS+ compared to CS- across fear extinction, whereas men do not. However, there were no overall sex differences in response to CS+ conditions, which again suggests caution in interpretation. This trend level finding and lack of overall sex differences accords with growing evidence that sex differences (and particularly effect of low oestrogen) is not as evident in fear extinction learning but is more prevalent in fear extinction recall which involves memory consolidation processes (Milad et al., 2006; Milad et al., 2010; Zeidan et al., 2011).

An unexpected sex difference was observed within the significant three-way interaction in the early extinction phase. Pairwise comparisons revealed that males' SCR was higher than females' SCR to CS- (with no sex differences to CS+) on Trial 1 and this pattern was at trend level for Trial 4. It is possible that females had learnt that the CS- was a safety signal and had remembered this in the extinction phase, whereas males may have demonstrated greater uncertainty across conditions. This is an interesting finding, as this finding suggests that females show less fear responding to safety signal, whereas males show more fear responding and possibly more uncertainty to a safety signal.

### **Sex Differences in Extinction Recall**

The results of this study partially supported that hypothesis that women in the early follicular phase would display impaired fear extinction recall relative to men, which would be reflected by a greater recovery of fear. In the early extinction recall phase, we did not find any significant sex differences between the percent recovery of fear to the CS+, however we found a trend for a sex difference in the extinction late recall phase ( $\eta p^2 = .116$ ). Interestingly, when examining Figure 9 and Figure 10, mean differences were in the expected direction that women in the early follicular phase exhibited a greater recovery of fear, relative to men (70.14% vs 54.43% in early recall, 73.66% vs 48.88% for late), albeit not reaching significance. These results provided partial support for our hypotheses and for findings obtained by previous studies (Graham and Milad (2013), Milad et al. (2010) and Zeidan et al. (2011)), where females with low levels of oestrogen displayed impaired fear extinction at recall, relative to males, and females with high levels of oestrogen. Similar findings with oestrogen playing an important role in the facilitation of extinction memory have also been demonstrated in rat studies, with female rats in the metestrus phase, characterised by low levels of oestrogen, demonstrated impaired fear extinction recall, relative to male rats, and female rats with high oestrogen levels (Milad et al., 2009). It is possible that we did not find a significant difference between females in the early follicular phase and males in support of the previous studies (Graham & Milad, 2013; Milad et al., 2010; Zeidan et al., 2011), due to a limited sample size, which led to poor power. In addition, we included women on oral contraceptives due to considerable difficulty with participant recruitment (data was collected for over a 12 month period). The inclusion of women on contraceptives may have also reduced the effect size obtained in the study.

### **Limitations and Future Research**

Despite the contribution of the present study to the area of fear conditioning and extinction, the present study has a number of potential limitations. One of the key limitations of the present study is the small sample size, which led to this study being considerably underpowered. This may be attributed to the difficulty in the recruitment of participants, especially as there were several requirements that the participant needed to meet in order to participate, e.g., age restrictions, no contraception in women other than the contraception pill, and no psychological, neurological or cardiovascular disease.

The difficulty in recruitment also limited the scope of the study, as females were only tested in the early follicular phase of the menstrual cycle, where oestrogen levels are typically low. The original aim of this project was to recruit and test women in both the low oestrogen (early follicular) and high oestrogen (late follicular) phases of the menstrual cycle, which would have provided a more direct and definitive test of the impact of oestrogen on fear extinction. In order to replicate the findings obtained from previous studies examining the sex differences in fear extinction recall, conducted by Graham and Milad (2013), Milad et al. (2010), and Zeidan et al. (2011), future research should endeavour to investigate other phases of the menstrual cycle, such as the late follicular phase, where oestrogen levels are high.

In addition, whilst all women were tested within 1-7 days of commencing menstruation, thus ensuring they were in the early follicular phase, oestrogen levels in participants in the current study were not confirmed with salivary samples<sup>3</sup>. It is recommended that for future studies, confirmation of oestrogen levels should be included, especially when examining other phases of the menstrual cycle.

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<sup>3</sup> Salivary samples were obtained, however not analysed as it was not within the scope of the thesis.



A further limitation was the inclusion of women on oral contraception in the study, necessitated due to participant recruitment difficulties. The initial recruitment of women not on contraception was attempted over a period of seven months, but proved too difficult and necessitated including women in the sugar pill phase of the contraceptive pill. In addition, research has also indicated that women on the sugar pill phase of the contraceptive pill have comparative levels of extinction recall relative to women in the early follicular phase of their menstrual cycle (Graham & Milad, 2013). In the current study, females in the early follicular phases consisted of females who did and did not take oral contraceptives (8 females without oral contraceptives and 7 females on oral contraceptives), which previous research has indicated that both groups demonstrate similar levels of oestrogen (Pluchino et al., 2009). Unfortunately, we did not have sufficient numbers of participants to compare females with and without contraception statistically. However, it may be helpful for future research to explore the impact of oral contraceptives and other forms of contraception, such as implants or intrauterine devices, on fear conditioning and fear extinction. This is particularly important as approximately 45% of women aged between 18 and 44 use some form of contraception in Australia (Gray & McDonald, 2010).

To date, no studies have examined fear conditioning and fear extinction across the different phases of the menstrual cycle in females, by utilising a within-subjects design. This may allow a more nuanced understanding of differences in fear conditioning and fear extinction with variations in sex hormones, as the luteal phases are associated with high progesterone and high oestrogen (mid luteal phase), and high progesterone and low oestrogen (late luteal phase), and the effects of oestrogen on extinction recall may vary in the context of high and low progesterone (Wegerer,

Kerschbaum, Blechert, & Wilhelm, 2014). However, a potential barrier to this would be the challenge of identifying valid ways of repeated testing of fear extinction recall in a within subjects design.

### **Theoretical and Clinical Implications**

Although the current study has important limitations that must qualify conclusions, they do support a growing body of research that has potentially important clinical implications. Various epidemiological studies have consistently demonstrated that women are more likely to have an anxiety disorder than men (Kessler et al., 2005; Kinrys & Wygant, 2005; Pigott, 2003). In addition, studies have also indicated periods in which oestrogen exists at low levels, such as premenstrual and postpartum periods, are associated with a worsening of symptoms and mental state (Douma et al., 2005; Östlund, Keller, & Hurd, 2003). It is possible that the findings of the current study may influence the treatment of individuals with anxiety disorders, so that exposure treatment (which is based on the principles of extinction learning) is focused on periods where women demonstrate higher levels of oestrogen, i.e., during the late follicular phase or mid-luteal phase, to facilitate extinction learning. Additionally, given that periods in which low levels of oestrogen, i.e., the early follicular phase, are associated with poor extinction memory recall, it is possible that treatment of anxiety disorders may be facilitated by providing individuals with an endogenous administration of oestrogen prior to extinction training. Initial evidence in support of this hypothesis is seen in a study conducted by Graham and Milad (2013) where women in the early follicular phase were administered oestrogen 30 minutes prior to extinction and displayed enhanced extinction recall, relative to women given a placebo. These promising results with oestrogen may be trialled in the future with individuals undergoing anxiety

treatment, to determine whether these results are obtained in a clinical setting. It is possible that treatment for anxiety disorders could also be successfully obtained with fewer sessions as well, if it is found that oestrogen facilitates extinction recall.

## **Conclusion**

The aim of the current study was to replicate the findings obtained by previous authors, by investigating sex differences in fear conditioning, fear extinction, and extinction recall, in particular in women with low levels of oestrogen. No sex differences were observed in fear acquisition or late extinction, but males displayed greater SCR to the CS- in early extinction, which may reflect greater arousal to uncertainty. There was a trend for females to continue to have greater SCR to the CS+ compared to the CS- throughout early extinction, which was not apparent in males. In addition, females displayed a trend for greater SCR responses during extinction recall compared to men. Although there were significant limitations in terms of power, sample size and composition, the findings of the current study provide partial support for previous studies that have found poor extinction recall in women with low levels of oestrogen. It is possible that oestrogen may have an important role in consolidating extinction memories, however further research is required with larger samples, and examining all menstrual phases in addition to the impact of oral contraceptives, to investigate the role of oestrogen in facilitating extinction recall.

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## Appendix A1

## Depression, Anxiety and Stress Scale

DASS <sub>21</sub>		Name:		Date:	
Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.					
The rating scale is as follows:					
0 Did not apply to me at all					
1 Applied to me to some degree, or some of the time					
2 Applied to me to a considerable degree, or a good part of time					
3 Applied to me very much, or most of the time					
1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

## Appendix A2

## Difficulties in Emotion Regulation Scale

**Please indicate how often the following statements apply to you by writing the appropriate number from the scale below on the line beside each item:**

---

1-----	2-----	3-----	4-----	5-----
almost never	sometimes	about half the time	most of the time	almost always
(0-10%)	(11-35%)	(36-65%)	(66-90%)	(91-100%)

---

- \_\_\_\_\_ 1) I am clear about my feelings.
- \_\_\_\_\_ 2) I pay attention to how I feel.
- \_\_\_\_\_ 3) I experience my emotions as overwhelming and out of control.
- \_\_\_\_\_ 4) I have no idea how I am feeling.
- \_\_\_\_\_ 5) I have difficulty making sense out of my feelings.
- \_\_\_\_\_ 6) I am attentive to my feelings.
- \_\_\_\_\_ 7) I know exactly how I am feeling.
- \_\_\_\_\_ 8) I care about what I am feeling.
- \_\_\_\_\_ 9) I am confused about how I feel.
- \_\_\_\_\_ 10) When I'm upset, I acknowledge my emotions.
- \_\_\_\_\_ 11) When I'm upset, I become angry with myself for feeling that way.
- \_\_\_\_\_ 12) When I'm upset, I become embarrassed for feeling that way.
- \_\_\_\_\_ 13) When I'm upset, I have difficulty getting work done.

---

1-----	2-----	3-----	4-----	5
almost never	sometimes	about half the time	most of the time	almost always
(0-10%)	(11-35%)	(36-65%)	(66-90%)	(91-100%)

---

- \_\_\_\_\_ 14) When I'm upset, I become out of control.
- \_\_\_\_\_ 15) When I'm upset, I believe that I will remain that way for a long time.
- \_\_\_\_\_ 16) When I'm upset, I believe that I'll end up feeling very depressed.
- \_\_\_\_\_ 17) When I'm upset, I believe that my feelings are valid and important.
- \_\_\_\_\_ 18) When I'm upset, I have difficulty focusing on other things.
- \_\_\_\_\_ 19) When I'm upset, I feel out of control.
- \_\_\_\_\_ 20) When I'm upset, I can still get things done.
- \_\_\_\_\_ 21) When I'm upset, I feel ashamed with myself for feeling that way.
- \_\_\_\_\_ 22) When I'm upset, I know that I can find a way to eventually feel better.
- \_\_\_\_\_ 23) When I'm upset, I feel like I am weak.
- \_\_\_\_\_ 24) When I'm upset, I feel like I can remain in control of my behaviors.
- \_\_\_\_\_ 25) When I'm upset, I feel guilty for feeling that way.
- \_\_\_\_\_ 26) When I'm upset, I have difficulty concentrating.
- \_\_\_\_\_ 27) When I'm upset, I have difficulty controlling my behaviors.
- \_\_\_\_\_ 28) When I'm upset, I believe that there is nothing I can do to make myself feel better.
- \_\_\_\_\_ 29) When I'm upset, I become irritated with myself for feeling that way.
- \_\_\_\_\_ 30) When I'm upset, I start to feel very bad about myself.
- \_\_\_\_\_ 31) When I'm upset, I believe that wallowing in it is all I can do.
- \_\_\_\_\_ 32) When I'm upset, I lose control over my behaviors.

---

1-----	2-----	3-----	4-----	5
almost never	sometimes	about half the time	most of the time	almost always
(0-10%)	(11-35%)	(36-65%)	(66-90%)	(91-100%)

---

- \_\_\_\_\_ 33) When I'm upset, I have difficulty thinking about anything else.
- \_\_\_\_\_ 34) When I'm upset, I take time to figure out what I'm really feeling.
- \_\_\_\_\_ 35) When I'm upset, it takes me a long time to feel better.
- \_\_\_\_\_ 36) When I'm upset, my emotions feel overwhelming.

## Appendix A3

## Health Questionnaire

**Health Questionnaire:**

1. Are you taking medications? What and what dose?

---

2. How many drinks of caffeine did you have yesterday?

---

3. What time was your last drink of caffeine yesterday?

---

4. How much exercise do you typically do each day?

---

5. How many minutes of exercise did you do yesterday?

---

6. When did you do finish your exercise yesterday?

---

7. How many cigarettes did you smoke yesterday?

---

8. When was the last cigarette that you smoked yesterday?

---

9. How many alcoholic drinks did you have yesterday?

---

10. At what time was your last alcoholic drink yesterday?

---

11. How many alcoholic drinks do you typically have each week?

---

**12.** Have you ever had a head injury/loss of consciousness for more than 5 minutes?

YES/NO

**13.** Do you have any other neurological disorders?

(epilepsy/parkinsons/dementia)

YES/NO

**a.** If so, what and for how long have you had this?

---

**14.** Have you any problems with your mental health (including an anxiety disorder or depression?) If so, what problem and for how long?

---

**15.** Do you have any problems with your memory? If yes. What?

---



## Appendix B1

# Tasmanian Social Sciences Human Research Ethics Committee Amendment Approval Letter

Social Science Ethics Officer  
Private Bag 01 Hobart  
Tasmania 7001 Australia  
Tel: (03) 6226 2763  
Fax: (03) 6226 7148  
Human.ethics@utas.edu.au



HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

3 April 2014

Professor Kim Felmingham  
Psychology  
Private Bag 30

*Sent via email*

Dear Professor Felmingham

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT  
Ethics Ref: **H0012496 - Sex differences in fear extinction: The influence of cognitive variables**

- Change of investigators: addition of Masters student Annie To, removal of Hollie Blackley and Matthew Wade.
- Addition of a second testing session where participants will be asked to return 24 hours later and will undergo the extinction phase of the testing session again.
- Revised Information Sheet.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 1 April 2014.

*Yours sincerely*

Katherine Shaw  
Executive Officer  
Tasmania Social Sciences HREC

## Appendix B2

## Participant Information Sheet



Title: **Sex differences in fear extinction**

Date:

**Invitation**

*You are invited to participate in a research study examining the influence of hormones on fear extinction. This study will be carried out in the Cognitive Neuroscience (ERP) Laboratory at the School of Psychology, University of Tasmania (Hobart). This study is being conducted by Annie To (Masters student), supervised by Professor Kim Felmingham in partial fulfilment of the requirements of their postgraduate studies in the School of Psychology, University of Tasmania.*

***What is the purpose of this study?***

The purpose of this study is to investigate the influence of hormones on fear conditioning and extinction which are key processes thought to underlie the development and treatment of anxiety disorders. Recent evidence reveals that cognitive variables and sex may influence the rates of fear conditioning and extinction, but few studies have examined the influence of hormones.

***Why have I been invited to participate?***

You have been invited to participate as you are a psychology first year student and this project is being offered as part of research participation course credit. We are looking for volunteers between the ages of 18 and 55, who are not currently taking

any medication, and who have no history of psychiatric disorders. We will ask you to complete a questionnaire about these conditions before the experiment begins.

***What will I be asked to do?***

You will be asked to come in for two testing sessions at the University of Tasmania, the first will take approximately 60 minutes and the second (24 hours later) will take approximately 30 minutes. The study will be run in the Cognitive Neuroscience Laboratory in the School of Psychology. In the first session, you will be asked to sit in a quiet room and complete some questionnaires about your mood, beliefs and cognitive processing style. You will also be asked to fill in a medical history questionnaire, which will ask about the position that you are in your menstrual cycle and contraceptive use (if you are female). The study will also require taking saliva samples (collecting saliva in a small plastic tube). The samples will be examined by laboratory technicians to measure your current levels of oestrogen, progesterone, noradrenaline and cortisol. You will then complete a behavioural task which examines how your body arousal (sweat gland activity) reacts to a mild electrical stimulus that will be administered to your fingertips. You will first be asked to select a level of mild electrical stimulus that feels uncomfortable but not painful to you. This will be done by attaching a finger stimulator to your index finger and delivering the lowest level of electrical stimulus, the level of which will then be increased in small increments until you report that it feels uncomfortable but not painful. You will then be asked to complete the behavioural task. In this task, you will sit in front of a computer screen and small recording disks will be attached to your finger tips to measure your body arousal (via skin conductance). You will then be asked to watch the computer screen on which you will see different coloured circles (red, or blue) appear. Following the presentation of some of these coloured circles, you will

receive an electrical stimulus which will be set at the level which you have previously chosen. You will also be asked to provide ratings on how much you are expecting to receive the electrical stimulus in the task. The behavioural task will last approximately 15 minutes.

In the second session, you will be asked to provide a second saliva sample and then complete one part of the behavioural task again. This will involve having small recording disks and the finger stimulator to your fingers, and observing the coloured circles. In this second testing session, you will not receive electric shocks.

**What will happen to my sample after it has been tested?**

Your saliva sample will only be used for the purpose of this research study. The saliva samples you provide during the study will be destroyed at the completion of the study. Your saliva samples will not be used for genetic testing or disease markers.

**Will I be able to get my sample back if I want?**

No, your saliva sample will be destroyed following laboratory analysis.

**Will drug or biotechnology companies be able to use my sample for profit in future?**

No.

**How is this study being paid for?**

The study is being sponsored by a grant from the National Health and Medical Research Council.

***Are there any possible benefits from participation in this study?***

If you decide to participate in this research you will gain experience in research procedures and also some knowledge of underlying mechanisms of anxiety and exposure therapy. If you are enrolled in first year Psychology, you will also receive

research participation credit of 1 hour for your participation. Furthermore, you will be involved in research that may provide a platform to better understand the mechanisms and processes involved in the extinction of fear, and this may ultimately lead to more efficient and effective exposure treatments for anxiety disorders.

***Are there any possible risks from participation in this study?***

Prior to commencement of the study you will be asked to sign consent form which will evidence your agreement to participate. You may feel a small amount of arousal or discomfort from the mild electrical stimulus. However, we expect that this arousal or discomfort to be minimal as the level that is administered will have been selected by you to be uncomfortable but not painful. The technology used to administer this electrical stimulus is very safe and has been used in many previous studies with no adverse effect reported. There will be a researcher with you at all times, and you can discontinue the study at any time without penalty and it will not affect your relationship with the University of Tasmania or the School of Psychology.

***What if I change my mind during or after the study?***

Participation in this research is entirely voluntary. You may choose to withdraw from the study at any time without prejudice. Deciding to withdraw from this research at any time will not affect your academic standing in any way. You can also choose at this time to withdraw any data previously collected. Participants will be given copies of this information sheet and the statement of informed consent.

***What will happen to the information when this study is over?***

Your individual data will be treated confidentially, your name will be replaced by an ID number on all data. It will be kept in a locked cabinet or on password secured computers at the School of Psychology at the University of Tasmania for a period of

at least five years (with the exception of the medical questionnaires which will be destroyed on completion of the study).

***How will the results of the study be published?***

Following completion of the research, the data will be published. However, no participant will be personally identifiable in these publications as only group data will be published. A summary of the results of these experiments will be available on the university of Tasmania School of Psychology Web page at [www.scieng.utas.edu.au/psychol](http://www.scieng.utas.edu.au/psychol) or will be available by contacting the researchers.

***What if I have questions about this study?***

The researchers will be available after the testing session to answer any questions you may have. If you have any questions, or would like any additional information regarding this research please contact, [Annie To ato0@postoffice.utas.edu.au.](mailto:ato0@postoffice.utas.edu.au), or Prof Kim Felmingham at [Kim.Felmingham@utas.edu.au](mailto:Kim.Felmingham@utas.edu.au).

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email [human.ethics@utas.edu.au](mailto:human.ethics@utas.edu.au). The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number H0012496.

**Thank you for taking the time to consider this study.**

**If you wish to take part in it, please sign the attached consent form.**

**This information sheet is for you to keep.**

## Appendix B3

## Consent Form

*Participant Consent Form***Sex differences in fear extinction: The influence of cognitive variables.****Participant Consent Statement:**

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. Any questions that I have asked have been answered to my satisfaction.
5. I understand that the study requires me to attend the Cognitive Neuroscience laboratory at the School of Psychology where my arousal responses will be recorded whilst I view different coloured circles and receive a mild electrical stimulus to my fingers. I understand that I can set the level of this mild electrical stimulus to feel uncomfortable but not painful prior to the task. I understand I will be asked to provide a saliva sample to get estimates of estrogen and progesterone. I also understand that I will attend two sessions for this task – one will take approximately one hour and one two days later for 30 minutes.
6. I understand that I will be asked about recreational drug habits, use of prescription medication and my menstrual cycle and contraceptive use (if female). I also understand that I should indicate to their experimenter if I have sensitive skin and that I should request a rest if I become fatigued.
7. I understand that all research data will be treated as confidential. I agree that research data gathered for the study may be published provided that I cannot be identified as a participant.
8. I understand that my participation is voluntary and that I may withdraw from participation and/or withdraw my data at any time without prejudice to my academic standing

Participant's name: \_\_\_\_\_

Participant's signature: \_\_\_\_\_

Date: \_\_\_\_\_

**Investigator Statement**

I have explained this research and the implications of participation in it to this volunteer and I believe that the consent is informed and that she understands the implications of participation

Investigator's name: \_\_\_\_\_

Investigator's signature: \_\_\_\_\_

Date: \_\_\_\_\_



## Appendix C

Table C1

*Non-significant Interactions for SCR in the Habituation Phase*

			<i>Mean</i>	<i>F</i>	<i>df</i>	<i>p</i>	<i><math>\eta p^2</math></i>
Condition by sex	Male	CS+	.68	1.95	1, 25	.175	.072
		CS-	.72				
	Female	CS+	.59				
		CS-	.53				
Condition by trial	CS+	Trial 1	.77	0.40	3, 75	.756	.016
		Trial 2	.69				
		Trial 3	.55				
		Trial 4	.52				
	CS-	Trial 1	.75				
		Trial 2	.63				
		Trial 3	.58				
		Trial 4	.53				

Table C2

*Non-significant Interactions for SCR in the Acquisition Phase*

			<i>Mean</i>	<i>F</i>	<i>df</i>	<i>p</i>	$\eta p^2$
Condition by sex	Male	CS+	.80	0.02	1, 25	.885	.001
		CS-	.69				
	Female	CS+	.75				
		CS-	.62				
Condition by trial	CS+	Trial 1	.82	1.26	4, 100	.292	.048
		Trial 2	.63				
		Trial 3	.83				
		Trial 4	.74				
		Trial 5	.88				
	CS-	Trial 1	.70				
		Trial 2	.56				
		Trial 3	.69				
		Trial 4	.71				
		Trial 5	.62				
Trial by sex	Male	Trial 1	.73	0.84	4, 100	.504	.032
		Trial 2	.63				
		Trial 3	.80				
		Trial 4	.74				
		Trial 5	.83				
	Female	Trial 1	.79				
		Trial 2	.56				
		Trial 3	.72				

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Trial 4	.70
Trial 5	.67

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Table C3

*Summary of Breakdown two-way ANOVA for the Sex by Condition interaction at each Trial in the Early Extinction phase*

			<i>Mean</i>	<i>F</i>	<i>df</i>	<i>P</i>	$\eta p^2$
Trial 1	Sex	Male	.89	1.97	1, 25	.173	.073
		Female	.70				
	Condition	CS+	.80	0.00	1, 25	.998	0.00
		CS-	.80				
	Sex by Condition			9.47	1, 25	.005	.275
Trial 2	Sex	Male	.78	.83	1, 25	.370	.032
		Female	.67				
	Condition	CS+	.73	.02	1, 25	.883	.001
		CS-	.72				
	Sex by Condition			.19	1, 25	.663	.008
Trial 3	Sex	Male	.59	2.98	1, 25	.097	.106
		Female	.41				
	Condition	CS+	.48	.32	1, 25	.578	.013
		CS-	.52				
	Sex by Condition			.98	1, 25	.331	.038
Trial 4	Sex	Male	.72	4.03	1, 25	.056	.139
		Female	.48				
	Condition	CS+	.61	.06	1, 25	.809	.002
		CS-	.59				
	Sex by Condition			.42	1, 25	.525	.016
Trial 5	Sex	Male	.60	.71	1, 25	.409	.027

	Female	.51				
Condition	CS+	.60	1.35	1, 25	.257	.051
	CS-	.51				
Sex by Condition			3.22	1, 25	.085	.114

Table C4

*Summary of non-significant post-hoc pairwise comparisons for the Trial by Condition interaction for females in the Early Extinction phase*

		Mean	p	95% CI
Trial 2	CS+	.65	.832	[-.29, .23]
	CS-	.68		
Trial 3	CS+	.43	.746	[-.16, .21]
	CS-	.40		
Trial 4	CS+	.51	.386	[-.10, .23]
	CS-	.44		

Table C5

*Non-significant Interactions for SCR in the Late Extinction Phase*

			<i>Mean</i>	<i>F</i>	<i>df</i>	<i>p</i>	$\eta p^2$
Condition by sex	Male	CS+	.70	0.09	1, 25	.763	.004
		CS-	.69				
	Female	CS+	.51				
		CS-	.53				
Condition by trial	CS+	Trial 1	.67	1.64	2.94, 73.38	.170	.062
		Trial 2	.59				
		Trial 3	.62				
		Trial 4	.53				
		Trial 5	.60				
	CS-	Trial 1	.66				
		Trial 2	.61				
		Trial 3	.52				
		Trial 4	.70				
		Trial 5	.55				
Trial by sex	Male	Trial 1	.80	0.89	2.29, 57.36	.474	.025
		Trial 2	.70				
		Trial 3	.59				
		Trial 4	.71				
		Trial 5	.67				
	Female	Trial 1	.53				

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	Trial 2	.49				
	Trial 3	.56				
	Trial 4	.53				
	Trial 5	.48				
Condition by Sex by Trial			0.67	2.94,	.617	.026
				73.38		

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